PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation Number: 1113
Robert Bayer	Examiner: Rao, Manjunath
Application No.: 09/855,320	Technology Center/Art Unit: 1652
Filed: May 14, 2001	DECLARATION OF DR. ROBERT BAYER UNDER 37 C.F.R. § 1.132
For: IN VITRO MODIFICATION OF GLYCOSYLATION PATTERNS OF RECOMBINANT GLYCOPEPTIDES	"THOMAS DECLARATION"
Customer No.: 43850	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Robert Bayer, Ph.D. declare as follows:

1. I am Senior Director of Research at Neose Technologies, Inc. My duties include directing the research operations of approximately 20 scientists at Neose's San Diego facility. Among these research operations are Neose's GlycoAdvance collaborations. GlycoAdvance is the name of our *in vitro* glycosylation technologies. I have over 14 years experience in this technology area. My *Curriculum Vitae* is attached as Exhibit 2A.

2. I am an inventor of the subject matter claimed in U.S. Patent Application No. 09/855,320 entitled "*In Vitro* Modification of Glycosylation Patterns of Recombinant Glycopeptides" ("the '320 Application"). I am familiar with the material contained in this application.

3. The '320 Application discloses glycopeptides with a "substantially uniform glycosylation pattern" prepared through contacting a glycopeptide having a glycosyl acceptor with a glycosyltransferase and a glycosyl donor moiety. The '320 Application further discloses and also claims a glycopeptide with a "substantially uniform fucosylation pattern" prepared by contacting a glycopeptide having a fucosyl acceptor with a fucosyltransferase and a fucosyl donor moiety.

4. In an earlier filed Declaration by Dr. David Zopf ("Zopf Declaration"), a scientific paper, Thomas, L.J. *et al.*, *Glycobiology* **14(10)**: 883-893 (2004) ("Thomas"), was presented. Thomas was the result of a collaboration between the assignee of the '320 Application and Avant Immunotherapeutics, and is attached as Exhibit 2B.

5. I am submitting this declaration to clarify the substantial identity between the methods and results of the '320 Application and those of the Thomas reference.

6. The starting material in an example of the '320 Application and in Thomas were substantially identical. See paragraph 13.

7. The starting material in an example of the '320 Application and in Thomas were submitted to substantially identical fucosylation conditions. The only difference between the examples disclosed in the two documents is the ratio of fucosyl donor to fucosyl acceptor substrate. In the '320 Application, this ratio is 14:1 (donor:acceptor); in Thomas, this ratio is 7:1 (donor:acceptor). See paragraph 14.

8. Because substantially the same starting materials were submitted to substantially the same fucosylation conditions, one of skill in the art would appreciate that the products of the method of the '320 Application and those of Thomas are substantially the same.

9. The '320 Application claims a composition comprising a glycopeptide having a "substantially uniform fucosylation pattern."

10. The term "substantially uniform glycosylation pattern" is defined on page 15, lines 1-3 and 12-15 of the '320 Application as follows:

> A "substantially uniform glycoform" or a "substantially uniform glycosylation pattern," when referring to a glycopeptide species, refers to the percentage of acceptor moieties that are glycosylated by the glycosyltransferase of interest (*e.g.*, fucosyltransferase). . .
>
> The term "substantially" in the above definitions of "substantially uniform" generally means at least about 60%, at least about 70%, at least about 80%, or more preferably at least about 90%, and still more preferably at least about 95% of the acceptor moieties for a particular glycosyltransferase are glycosylated.

According to this definition, a minimum of 60% of the glycosyl acceptor moieties on a glycopeptide must be glycosylated in order for the glycopeptide to possess a "substantially uniform glycosylation pattern". Therefore, for the specific case of fucose, a minimum of 60% of the fucosyl acceptor moieties on a glycopeptide must be fucosylated in order for the glycopeptide to possess a "substantially uniform fucosylation pattern".

11. The 7:1 fucosylation conditions in Thomas yielded a product with a "substantially uniform fucosylation pattern". Therefore, the products subjected to the 14:1 fucosylation conditions of the '320 Application also possess a "substantially uniform fucosylation pattern". See paragraphs 16-25.

12. To the extent that the fucosylation conditions differ, the fucosylation conditions of the '320 Application would not be expected to reduce the total percentage of fucosylation, because there are more fucose donors present in the reaction mixture.

The starting materials in the '320 Application and in Thomas were substantially identical.

13. In both Thomas and the '320 Application, the starting material was sCR1-S. In both Thomas and the '320 Application, this starting material was produced *in situ* from a sialylation reaction. The similarity in the starting materials was revealed through fluorophore-assisted carbohydrate electrophoresis gel ("FACE gel") analysis. The FACE gel analysis of the

starting material for Thomas was disclosed in lane 3 of Figure 1. The FACE gel analysis of the starting material for the '320 Application was disclosed in the 'sialylated' lane of Figure 3. The band patterns in both lane 3 and the 'sialylated' lane were the same. The higher of the two bands was a monosialylated glycan product (DP = 7) and the lower of the two bands was a disialylated glycan product (DP = 6.2). The structures of these glycan products are attached as part of Exhibit 2C. The monosialylated glycan product is structure A in Exhibit 2C while the disialylated glycan product is structure B of Exhibit 2C.

The same starting materials were subjected to substantially identical fucosylation conditions in the methods of Thomas and in the methods of the '320 Application.

14. Substantially identical fucosylation conditions are disclosed in Thomas and the '320 Application. These conditions are attached as Exhibit 2D. Thomas and the '320 Application disclose that the reaction temperatures as well as concentrations of fucose acceptors (sCR1-S), fucose donors (GDP-fucose) and fucosyltransferases (FT-VI) are the same. In addition, the ratio of fucosyltransferase to fucose acceptor (FT-VI : sCR1-S) is the same for Thomas (0.02 U FT-VI/mg sCR1-S) as for the '320 Application (0.02 U FT-VI/mg sCR1-S). The main difference between the two reaction conditions lies in the ratio of fucose donor to fucose acceptor. For Thomas, this ratio is 7:1. For the '320 Application, this ratio is 14:1. In other words, the method of the '320 Application utilizes a 2-fold excess of fucose donor (per fucose acceptor) as compared to Thomas. One of skill in the art would expect that more fucose donors would not reduce the total percentage of fucosylation. Therefore, if the 7:1 fucose donor : fucose acceptor fucosylation conditions of Thomas produce a "substantially uniform fucosylation pattern", then the 14:1 fucose donor : fucose acceptor fucosylation conditions of the '320 Application will also produce a "substantially uniform fucosylation pattern".

The products in the '320 Application and in Thomas are substantially identical.

15. The similarity in the products in Thomas and the '320 Application were revealed through FACE gel analysis. The FACE gel analysis of the products in Thomas was disclosed in lane 4 of Figure 1. The FACE gel analysis of the products in the '320 Application was disclosed

in the 'sialylated and fucosylated' lane of Figure 3. In both lane 4 and the 'sialylated and fucosylated' lane, a band was not present at DP 6.2, indicating the consumption of the unfucosylated, disialylated starting material. In both lane 4 and the 'sialylated and fucosylated' lane, one band was visible slightly below DP 7, with trace bands at higher DP values. The band contained a difucosylated, disialylated product. This product is structure D in Exhibit 2C.

The 7:1 donor : acceptor fucosylation conditions in Thomas yielded a product with a "substantially uniform fucosylation pattern".

16. The product of the 7:1 donor : acceptor fucosylation conditions in Thomas was subjected to HPLC and MALDI-TOF-MS analysis (Thomas, p. 884, column 1). The HPLC results are presented in paragraph 18. The MALDI-TOF-MS results are used in paragraphs 18-25 to determine whether the glycopeptide products of Thomas possess a "substantially uniform fucosylation pattern."

17. The HPLC analysis yielded monosaccharide content information, which was reported in the "sCR1-S/F" column in Table I of Thomas. According to structure D of Exhibit 2C, one of skill would expect the ratios of glucosamine: galactose: mannose: fucose: sialic acid to be 4: 2: 3: 3: 2. The reported relative amounts of these monosaccharides are 48: 27: 35: 39: 28, which reduces to 4: 2.3: 3: 3.3: 2.3. These experimental values correlate well with expected values.

18. The MALDI-TOF-MS analysis yielded molecular weight information about the fucosylation reaction products, which was reported in Figure 7C of Thomas. This molecular weight information was then converted into product percentages which are reported in Table III of Thomas.

19. Whether a glycopeptide has a "substantially uniform fucosylation pattern" was determined by dividing the total number of fucosylated acceptor sites by the total number of potential fucose acceptor sites. Paragraphs 20-23 detail the percentage of glycans that have one, two, three, or four acceptor sites, and the percentage of the acceptor sites that are fucosylated.

These percentages are then used in paragraph 24 to determine the total percentage of fucosylation, or whether a glycopeptide has a "substantially uniform fucosylation pattern".

20. Calculations for glycans with one acceptor site. The percentage of one-acceptor glycans having no fucose is 0.43 + 0.7 + 3.47 = 4.60. The percentage of one-acceptor glycans having one fucose is 0.95 + 1.15 + 7.07 = 9.17. The total percentage of glycans with one-acceptor glycans is (4.60 + 9.17) = 13.77. Of this number, 9.17/13.77 = 67% are fucosylated.

21. Calculations for glycans with two acceptor sites. The percentage of two-acceptor glycans having no fucose is 1.06. The percentage of two-acceptor glycans having one fucose is 1.28 + 2.93 + 17.26 + 0.13 = 21.60. The percentage of two-acceptor glycans having two fucoses is 0.58 + 6.12 + 51.24 + 0.82 = 58.76. The total percentage of glycans with two-acceptor glycans is (1.06 + 21.60 + 58.76) = 81.42. Of this number, 85.4% are fucosylated, as shown below.

$$\frac{(1.06)\times 0+(1.28+2.93+17.26+0.13)\times 1+(0.58+6.12+51.24+0.82)\times 2}{(81.42\times 2)}=85.4\%$$

22. Calculations for glycans with three acceptor sites. The percentage of three-acceptor glycans having no fucose is 0.16. The percentage of three-acceptor glycans having one fucose is 0.38. The percentage of three-acceptor glycans having two fucoses is 1.51 + 0.75 = 2.26. The percentage of three-acceptor glycans having three fucoses is 0.76. The total percentage of glycans with three-acceptor glycans is 3.55. Of this number, 67% are fucosylated, as shown below.

$$\frac{(0.16)\times 0+(0.38)\times 1+(1.51+0.75)\times 2+(0.75)\times 3}{(3.55\times 3)}=67\%$$

23. Calculations for glycans with four acceptor sites. The percentage of four-acceptor glycans having no fucose is 0.18. The percentage of four-acceptor glycans having one fucose is 0.35. The percentage of four-acceptor glycans having two fucoses is 0. The percentage of four-

acceptor glycans having three fucoses is 0. The total percentage of glycans with four-acceptor glycans is 0. The total percentage of glycans with four-acceptor glycans is 0.53. Of this number, 16% are fucosylated, as shown below.

$$\frac{(0.18)\times 0+(0.35)\times 1+(0)\times 2+(0)\times 3+(0)\times 4}{(0.53\times 4)}=16\%$$

24. Based on the percentages of paragraphs 20-23, the total percentage of fucosylation by Thomas is 83%, as shown below.

$$\frac{(13.77)\times 0.67+(81.42)\times 0.854+(3.55)\times 0.67+(0.53)\times 0.16}{(13.77+81.42+3.55+0.53)}=83\%$$

Since the total percentage of fucosylation is at least 60%, the glycopeptides produced by the methods of Thomas possess a "substantially uniform fucosylation pattern."

25. As mentioned in paragraphs 7 and 14, the fucosylation conditions between Thomas and the '320 Application are the same except that the method of the '320 Application utilizes a 2-fold excess of fucose donor (per fucose acceptor) as compared to Thomas. One of skill in the art would expect that more fucose donors would not reduce the total percentage of fucosylation. Therefore, since the 7:1 donor : acceptor fucosylation conditions of Thomas yield a glycopeptide with a "substantially uniform fucosylation pattern," the 14:1 donor : acceptor fucosylation conditions of the '320 Application also yield a glycopeptide with a "substantially uniform fucosylation pattern".

26. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: June 3 2005

Robert Bayer, Ph.D.

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